



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE APPLICATION OF :  
Kazuhiro YOSHINAGA :EXAMINER: HENRY, MICHAEL C  
SERIAL NO.: 10/579,978 :  
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FOR: EFFICIENT PRODUCTION :  
METHOD OF ASCOPYRONE P

DECLARATION UNDER 37 C.F.R. 1,132

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ALEXANDRIA, VIRGINIA 22313

I, Kazuhiro Yoshinaga, am a doctor of agriculture and one of the inventors of the present invention.

I have a good grasp of the method of preparing Ascopyrone P developed by Ahmad (The Swedish University of Agricultural Sciences, Sweden, pages 1-34, 1995). I have conducted the following experiments to demonstrate that the production yield of Ascopyrone P in the method is very low and that Ascopyrone P cannot be produced by the method at an industrially significantly high yield as seen in the present invention.

Experimental method  
Runs 1-4

Zero point three five milliliters of 1 M sodium hydroxide was added to 0.35 mL of an aqueous solution containing 57 mg/mL 1,5-anhydro-D-fructose, the resulting solution was heated at 25°C, 50°C, 75°C and 100°C for 5 minutes, 0.35 mL of 1 M HCl was added to neutralize it, and further the neutralized solution was diluted with water to 100 times to determine the quantity of the synthesized Ascopyrone P by high-pressure liquid chromatography (HPLC). The pH of the aqueous solution obtained by adding 0.35 mL of 1 M sodium hydroxide to 0.35 mL of an aqueous solution containing 57 mg/mL 1,5-anhydro-D-fructose was 13.

#### Run 5

Zero point three five milliliters of water was added to 0.35 mL of an aqueous solution containing 57 mg/mL 1,5-anhydro-D-fructose and heated at 50°C for 5 minutes (the temperature condition of Run 2 at which the conversion rate of 1,5-anhydro-D-fructose to Ascopyrone P is the highest, out of Runs 1-4), and then 0.35 mL of water was added, and the resulting solution was diluted with water to 100 times to determine the quantity of the synthesized Ascopyrone P by HPLC.

#### Run 6

Two milliliters of an aqueous solution containing 20 mg/mL 1,5-anhydro-D-fructose was heated at 70°C for 1 hour, and 1 mL of water was added to the solution to determine the quantity of the synthesized Ascopyrone P by HPLC.

#### Results and Considerations

Run 1 corresponds to the working experiment of the method disclosed by Ahmad. Runs 2, 3 and 4 correspond to experiments which were carried out at higher temperatures (50°C, 75°C and 100°C) than the temperature (25°C) of the method disclosed by Ahmad.

The conversion rate of 1,5-anhydro-D-fructose to Ascopyrone P obtained in Runs 1-4 are shown in Table A.

Table A

Run No.	Reaction temperature (°C)	Conversion of 1,5-anhydro-D-fructose to Ascopyrone P (%)	ratio to the yield of Ascopyrone P of Run No.1
1	25	2.3	1
2	50	7.4	3.1
3	75	4.2	1.8
4	100	0.05	0

In the experiment which was conducted under an alkaline condition shown by Ahmad, it is understood that the highest conversion rate was obtained at a reaction temperature of 50°C and the conversion rate of 1,5-anhydro-D-fructose to Ascopyrone P was very low at 100°C.

Run 5 corresponds to an experiment shown in Ahmad which was conducted under a neutral condition. In the experiment of Run 5, the conversion rate of 1,5-anhydro-D-fructose to Ascopyrone P was 0.01%.

Finally, Run 6 corresponds to an experiment in which an aqueous solution of 1,5-anhydro-D-fructose was heated at the temperature (70°C) disclosed by Elsser et al. In the experiment of Run 6, the conversion rate of 1,5-anhydro-D-fructose to Ascopyrone P was very low. That is, it is understood that even when a higher temperature than the temperature disclosed by Ahmad and the temperature (70°C) disclosed by Elsser et al. are employed, Ascopyrone P is rarely produced under the neutral condition.

The undersigned declares further that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of this application or any patent issuing thereon.

Further declarant saith not.

吉永 一浩

KAZUHIRO YOSHINAGA

5/28/2008

Date